A QUANTITATIVE EXAMINATION OF OVARIAN GROWTH IN THE WHITE-CROWNED SPARROW[1]

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In an earlier paper in this journal (Farner and Wilson, 1957) it was demonstrated that testicular growth in *Zonotrichia leucophrys gambelii* induced by stimulatory daily photoperiods of constant duration is approximately a logarithmic function of time from resting testicular weight until a weight of 200–250 mg. is attained. It was demonstrated further that the slope represented by the logarithmic growth-rate constant (k) has an approximately linear relationship to the duration of the daily photoperiod (p) over a wide range of values (*ca.* 9 to 18 hours) of p. Subsequently, a much greater accumulation of unpublished data has confirmed these relationships. Similar relationships have been demonstrated for *Chloris chloris, Fringilla montifringilla,* and *Fringilla coelebs* by Dolnik (1963), and apparently exist also in *Passer domesticus* (Bartholomew, 1949; Farner and Wilson, 1957; Farner, 1964; Vaugien, 1952, 1954, 1959; Middleton, 1965) and in *Coturnix coturnix japonica* (Mather and Wilson, 1964; Tanaka *et al.*, 1965; Follett and Farner, unpublished), although, as yet, the precise functional relationships of k to p are not known for the latter two species.

At the time of our investigation of the quantitative relationships between p and k in testicular growth in *Zonotrichia leucophrys gambelii,* we became aware of a basic photoperiodic control in the annual ovarian cycle. The much slower development of our knowledge of the role of the photoperiodic mechanism in the control of the ovarian cycle involves several factors. Prominent among these is the obviously slower and more variable growth rate of the ovary under photoperiodic stimulation. Because of this it has always been essential to use males in photoperiodic experiments in which quantitative data on response rates are essential. Also, contrary to the situation in males, complete gonadal development does not occur in captive females, regardless of photoperiodic regime (Farner, 1959, 1964). Furthermore, the accumulation of data on the ovarian cycle, both in the field and in the laboratory, has been necessarily at a much slower rate because of the very low fraction of females in the wintering flocks in the Snake River Canyon (Mewaldt

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and Farner, 1953), a major source of our experimental birds. However, we have now accumulated sufficient data to permit some quantitative characterization of photoperiodically induced ovarian growth. It is the function of this paper to present these data, together with our analyses and interpretations thereof.

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Materials and Methods

The experimental birds were captured with mist nets from migrating flocks in the vicinity of Pullman in autumn, and from a wintering population in the nearby Snake River Canyon. They were held in large outdoor aviaries until the end of January. At that time they were moved indoors and placed two or three per cage into small cages (41 × 22 × 26 cm.) on 8-hour daily photoperiods (8L 16D). Light was provided by incandescent lamps at an intensity of at least 400 lux. Room temperature was maintained at 18–19° C. Food, consisting of a vitamin- and mineral-enriched chick-starter mash, and water were freely available at all times. Each bird was weighed weekly, at the same time of day, to the nearest 0.1 g. Sex was determined by laparotomy; only first-year females were used in the experiment.

Six weeks after the birds were brought indoors, 10 were killed to obtain resting ovary weights. Of the remaining birds, 10 were retained as controls on 8L 16D and 50 were placed on 20-hour daily photoperiods (20L 4D) by extending the termination of the photoperiod by one 12-hour increment. The latter group was sampled at selected intervals during the next seven weeks, at the end of which time all remaining birds were killed.

Ovaries were fixed in a solution of 10 parts acetic acid, 10 parts formalin, 30 parts 95% ethanol, and 50 parts water for five days and then transferred to 70% ethanol for five additional days. They were then carefully debrided of extraneous tissues and weighed on a torsion balance. Anterior pituitary glands were removed immediately after killing and placed in dry acetone. The acetone was changed twice during the ensuing four days, after which the glands were air-dried and then stored *in vacuo* over phosphorus pentoxide at 0° C.

For interpretation of our laboratory data with respect to the natural ovarian cycle we have used data on ovarian weights from birds taken in the field in winter in the Snake River Canyon, during spring migration in the vicinity of Pullman, and during the breeding season in the vicinity of Fairbanks, Alaska. These birds were obtained by shooting or with mist nets. Treatment of the ovaries was similar to that for the experimental birds.

Gonadotropin was assayed by a modification of the method of Breneman *et al.* (1962), the details of which are being published elsewhere. In essence, day-old chicks were injected subcutaneously with either a homogenized pituitary extract from white-crowned sparrows or with a standard LH preparation (NIH-LH-S7);

six hours thereafter they received a dose (1.2 μc.) of carrier-free P^{32}. Eighteen hours later all animals were killed; the testes were removed, blotted and weighed to the nearest 0.1 mg. They were dried on aluminum planchets and counted with a Nuclear-Chicago gas-flow counter (Model D47) with automatic sample changer. Results were expressed as counts (minus background) per minute per mg. wet tissue.

Estimates were made with a balanced four-point factorial assay method. Eight to 10 chicks were used at each dose level. The results from an experiment were subjected to a full analysis of variance (Bliss, 1952); potency estimates are given, together with 95% confidence limits and the index of precision (λ). The latter shows the method to compare favorably with other methods of gonadotropin assay. A standard preparation of LH was employed since it allowed the estimate of potency from a single assay. In all cases glands from a number of birds were pooled for one assay; however, the method could be used to measure the gonadotropic content of individual glands.

Ovarian Weight as a Function of Time with a Constant Daily Photoperiod of Stimulatory Duration

An examination of the weights of the ovaries from the photostimulated group (Fig. 1) suggests that there is an approximately linear relationship between the logarithm of ovarian weight and time that may be expressed as

$$\log_{10} W_t = \log_{10} W_0 + kt,$$

where W_0 is ovarian weight in milligrams at the beginning of treatment with 20L 4D, W_t is the weight at t days, t is time in days on 20L 4D, and k is the logarithmic growth-rate constant in days^{-1}. It should be noted that k for ovarian growth in this experiment, 0.027 ± 0.003 days^{-1}, is much smaller than that, 0.111 ± 0.011, for a group of first-year males subjected to the same photoperiodic treatment at approximately the same time. This difference in laboratory-induced rates between the sexes is consistent with the generally observed slower rate of ovarian development under natural conditions in early spring (Table I) in which the increase in weight by the time of spring migration is approximately four-fold, compared with a 50-fold increase in testicular weight. If, as there is some reason to assume, the steady-state level of pituitary gonadotropin has a positive functional relationship to the rate of gonadotropin output, one must tentatively conclude that a given rate of output causes a more rapid testicular growth than ovarian growth (Table I). However, we cannot exclude the possibility that the relationship between pituitary gonadotropin level and the rate of output may be different in the two sexes.

The failure of ovarian growth to proceed beyond approximately 50 mg. in the birds subjected to 20L 4D is consistent with our long, although somewhat casual, experience with the behavior of females in captivity. In the 15 years in which we have used this species experimentally, only twice have we found single eggs in the outdoor aviaries; the production of eggs has been equally infrequent by females held in cages indoors (Merkel, 1963). Of interest here are a series of observations on levels of pituitary gonadotropins and gonadal development in spring in wild birds and birds held in large outdoor aviaries under

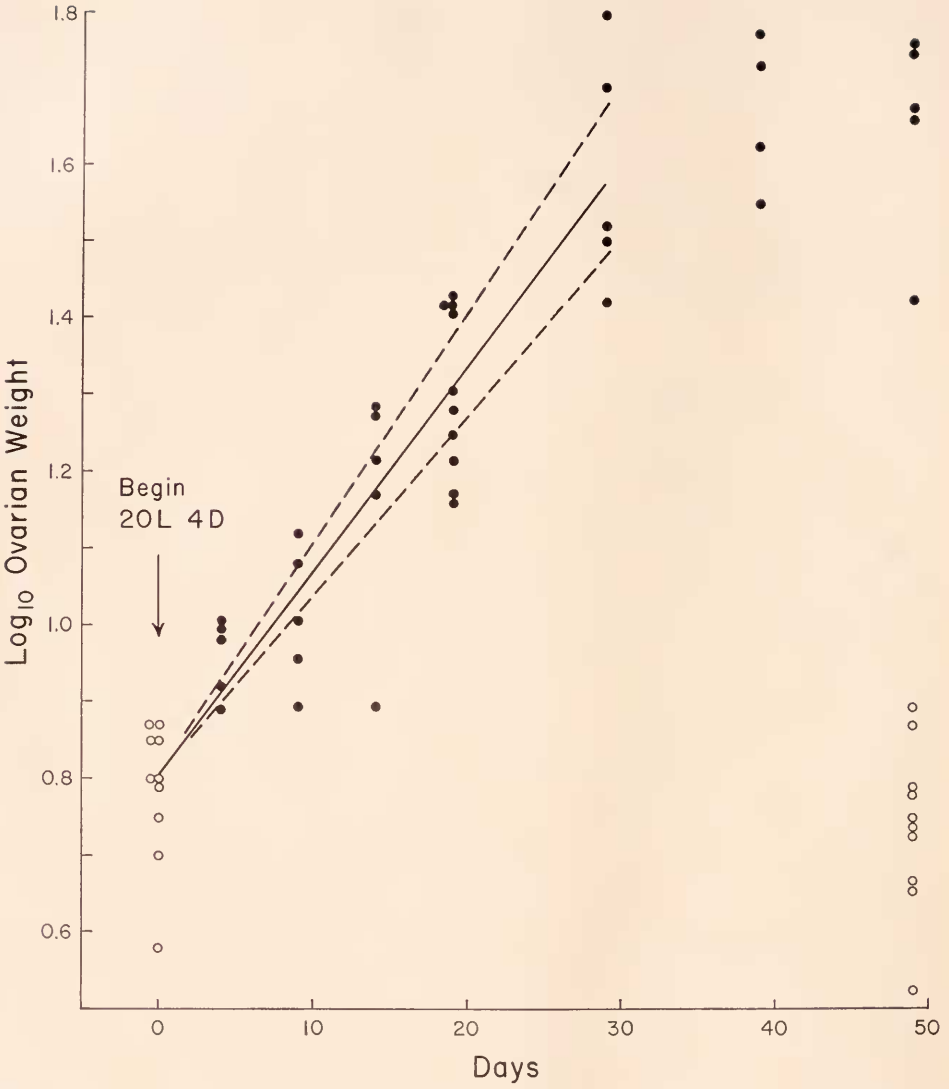

FIGURE 1. Ovarian weights (W) of first-year white-crowned sparrows as a function of time after a change in daily photoperiod from 8 (8L 16D) to 20 hours (20L 4D). Closed circles represent birds subjected to 20L 4D; open circles represent birds continued on 8L 16D.

natural conditions of temperature and photoperiod (Table I; also King *et al.*, in press). Migrating females taken in Pullman in early May had a mean ovarian weight of 18.6 ± 1.5 mg. and a pituitary gonadotropin content of 11.1 (8.4–14.7) μg./gland. For females taken late in May, shortly after arrival on the breeding grounds in the vicinity of Fairbanks, Alaska, the respective means were 326 ± 129 mg. and 9.8 (7.5–12.1) μg./gland. In contrast, the respective means

TABLE I

Relationship between gonadotropic potency of the anterior pituitary and relative gonadal weight (W) in male and female Zonotrichia leucophrys gambelii under natural conditions

Period	Male			Female		
	W* mg.	W/W_{max}	Pituitary gonadotropin**	W* mg.	W/W_{max}	Pituitary gonadotropin**
Mid-winter	1.1± 0.1	0.002	3.0 (2.6–3.6)	4.9± 0.5	0.015	3.1 (2.3–4.1)
Spring migration	47.7± 4.1	0.108	11.1 (9.4–14.1)	18.6± 1.5	0.057	11.1 (8.4–14.7)
Breeding ($W_{maximum}$)	442. ±31.	1.000	14.8 (13.5–16.1)	326. ±86.	1.000	9.8 (7.5–12.1)

* Mean and standard error.

** Microgram equivalents NIH-LH per gland; means with 95% confidence limits.

for females captured during spring migration and held in outdoor aviaries in Pullman until 3 June were 30.2 ± 2.8 mg. and 2.7 (1.6–4.4) μg./gland. The situation in the males was very different. For the spring migrants in Pullman the mean testicular weight was 47.7 ± 4.1 mg. and the pituitary gonadotropin 11.1 (9.4–14.1) μg./gland. The respective means for the breeding males taken in late May were 408 ± 14 mg. and 14.8 (13.5–16.1) μg./gland. However, males held in outdoor aviaries in Pullman until the first week of June showed only slight differences from the breeding sample, the means being 353 ± 16 mg. and 16.9 (12.6–22.8) μg./gland. Consistent with the observations of an approximately 50-mg. limit on ovarian growth in photoperiodic experiments are our unpublished observations of the failure of captive females to develop the brood patches and hypercalcemia that characterize the egg-laying period.

Consideration of the initial logarithmic growth of the ovary of birds subjected to long daily photoperiods, and of the behavior of the gonadotropic activity of the anterior pituitary in the experiment described herein and in unpublished experiments, leads to the conclusion that the gonadotropic function of the anterior pituitary in photostimulated females, until the 50-mg. ovary is attained, is essentially the same as in photostimulated males. The intriguing problem, of course, is the great difference between the sexes beyond this point. There is the possibility that, for reasons as yet unknown, the control system, in captive females, fails to maintain the level of gonadotropin release characteristic of the initial period of ovarian growth. There is also the possibility that growth and development of the ovary beyond the 50-mg. level requires a second gonadotropin which is not produced in captive birds. This can only be resolved by further investigations.

Equally interesting is the problem of the environmental etiology of the failure of the ovaries of captive females to develop beyond the 50-mg. level. Although we have no observations on which to base the suggestion, there is the possibility of some critical deficiency in the environment, such as Polikarpova (1940) was able to conclude from his experiments with *Passer domesticus*. In this species it appears, as in *Zonotrichia leucophrys gambelii*, that photoperiodic stimulation can cause the development of the ovary only up to a certain stage of development; further development comes only in the presence of an active male, and

complete development occurs only if nesting sites and nesting material are available. It is possible that in *Z. l. gambelii* the females have similar requirements, although our data suggest that active males do not exert such an influence on females in captivity. Whether or not active males in the migrating flocks, during the approximately three weeks that elapse between departure from the latitude of Pullman and arrival in the breeding areas in Alaska, can account for the difference in ovarian development between wild and captive birds is conjectural. Because of the partial separation of males and females in spring migration (King *et al.,* 1965) we suspect that an additional or alternate factor must be involved. It is possible also that conditions of captivity impose an inhibitory "fear reaction," such as that described by Phillips and van Tienhoven (1960) for female mallards (*Anas platyrhynchos*) and female pintails (*A. acuta*). Electrolytic lesions in the ventral medial archistriatum or occipito-mesencephalic tract resulted in the disappearance of "escape behavior" and in the development of the ovary (Phillips, 1964). Again it appears unlikely that such an explanation can be entirely adequate for *Z. l. gambelii* since development of the ovary up to the 50-mg. level proceeds normally under photoperiodic stimulation in caged birds. It is obvious that much additional research lies ahead in order to identify the environmental deficiency or inhibitor imposed by captivity on the females of *Z. l. gambelii.*

Non–Photoperiodic Ovarian Growth

An examination of ovarian weight in the first-year birds reveals, from the post-nestling stage until midwinter, an approximately linear increase (Fig. 2) that appears to be independent of the natural daily photoperiod. This conclusion is sustained by the observation of continued growth when birds are transferred to 8L 16D in midwinter to avoid the increasing natural daylength and subsequent photoperiodically induced ovarian growth. The regression line for all birds is shown in Figure 2; the equation for the line, based on 191 ovarian weights, is $W = 1.067 + 0.0186t$. Statistically, this slope is significantly greater than zero ($P \ll 0.001$).

An analysis of the ovarian weights for 76 adults indicates a similar but much smaller non-photoperiodic growth, the regression equation being $W = 4.693 + 0.0062t$. The slope of the line is of marginal significance ($P \leqslant 0.05$); however, the difference in slope between first-year females and the adults is highly significant ($P \ll 0.001$).

Little further can be said about this non-photoperiodic ovarian growth at this time. It would be of interest to learn how long it can continue under a regimen of short daily photoperiods. Insofar as the overall development of the ovary is concerned, the quantitative contribution of the non-photoperiodic phase is slight. Even first-year birds, at the rate shown in Figure 2, would require eight years, or about 5 times the mean life span, to attain the 50-mg. ovary characteristic of the migrant at the latitude of Pullman. This re-emphasizes the importance of the duration of the daily photoperiod in the basic timing of the ovarian cycle. Although the magnitude of the non-photoperiodic ovarian growth is small, it is important that it be recognized in photoperiodic experiments since serious errors can be introduced in the calculation of the logarithmic growth-rate constant by

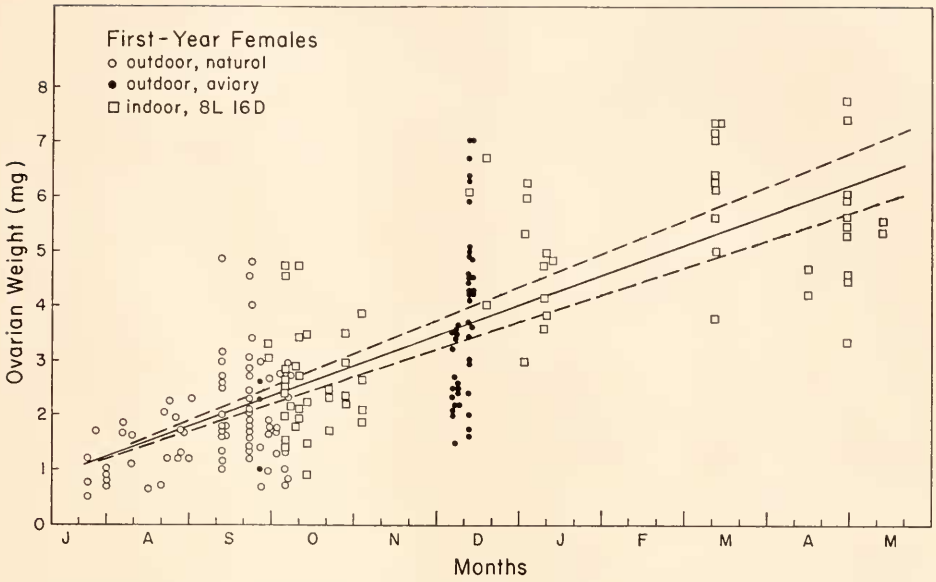

FIGURE 2. Non-photoperiodic growth of the ovary of the first-year white-crowned sparrow (*Zonotrichia leucophrys gambelii*). Ovarian weight is plotted as a function of time in months; during this period the birds did not receive stimulatory photoperiods. ○, birds taken from the field; ●, birds from outdoor aviaries under natural conditions of temperature and photoperiod; □, birds held indoors under 8L 16D. Field and outdoor aviary birds are not included beyond mid-January since natural photostimulation can first be detected in February.

failure to have a precisely determined W_0 for the date of initiation of long-day treatment.

Inspection of our data on testicular weights for first-year males has frequently prompted us to suspect the occurrence of a very slight non-photoperiodic growth through late fall and early winter. Despite the much larger amount of data available, our statistical analyses fail to sustain our suspicion. Certainly if such growth does occur, it is extremely slight. We find in our data from several hundred adult males no reason to suspect a non-photoperiodic testicular growth.

SUMMARY

1. Ovarian development in *Zonotrichia leucophrys gambelii* subjected to long daily photoperiods is a logarithmic function of time until a weight of approximately 50 mg. is attained. Although there is a slight non-photoperiodic ovarian growth, especially in first-year birds, it is clear that long daily photoperiods are essential for normal ovarian growth and constitute the basic environmental information used in the control of the ovarian cycle.

2. The photoperiodic control mechanism in the female differs from that of the male in that the logarithmic growth phase brings the ovary to only approximately one-tenth of its maximum weight, whereas the logarithmic growth phase of the testes in photostimulated males brings them to about one half of maximum

TABLE II

*Pituitary gonadotropin content in juvenile female Zonotrichia leucophrys gambelii exposed to long daily photoperiods**

Day of treatment	Mean gonadotropic** potency	95% Confidence limits	Lambda
0	7.9	5.8–10.3	0.157
4	5.4	3.8– 6.7	0.108
9	12.7	10.0–15.6	0.122
14	15.2	12.2–18.7	0.119
19	15.9	11.7–21.9	0.167
29	13 1	7.8–17.6	0.168
49	14.2	10.6–18.7	0.155
49 (final 8L 16D controls)	6.6	4.8– 8.2	0.116

* Twenty hours per day (20L 4D), after being held previously on short days (8L 16D).
** Microgram equivalents NIH-LH per gland; means with 95% confidence limits.

weight. In addition, the female differs from the male in that complete gonadal development can be induced only rarely in captivity with either artificial or natural photoperiodic stimulation, whereas sufficiently long daily photoperiods invariably result in the complete development of the testes of photosensitive males in captivity.

3. Until an ovarian weight of approximately 50 mg. is attained the gonadotropic performance of the anterior pituitary of the female in captivity appears to be similar to that of photostimulated males.

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